

The University of Chicago

THE EFFECT OF SEX HORMONES ON
BLOOD-CALCIUM AND INORGANIC
BLOOD-PHOSPHATE LEVELS

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PHARMACOLOGY

1931

By

HUBERT WHATLEY MARLOW

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THE EFFECT OF SEX HORMONES ON BLOOD-CALCIUM AND INORGANIC BLOOD-PHOSPHATE LEVELS^{1, 2}

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Changes in the level of blood and serum calcium have been associated from time to time with sex and reproduction. In fowl, pigeons and doves the results are very striking during the egg-laying periods but in mammals the reports are very conflicting and of doubtful significance.

The importance of the calcium intake in fowl during egg production, with normal shell formation, is well established (1, 2, 3, 4, 5, 6) and the hourly fluctuations in the blood-calcium values during laying periods have been beautifully demonstrated in fowl, pigeons and doves (6, 7, 8, 9, 10, 11). Fluctuations in the weights of ovaries, testes and thyroids with seasonal variations in blood-calcium in pigeons and doves were also noted by Reinhardt and Riddle (6). Macowan (10) also observed histological changes in the parathyroids of the laying pullet and showed that parathormone injections caused rises of 2 to 6.5 mg. of Ca per 100 cc. of serum in pullets but not in moulting hens or cockerels.

In the pregnant mammal we also recognize a change in calcium requirement, but the hourly fluctuations in calcium mobilization are not comparable to those existing in avian reproduction, hence changes in levels during pregnancy are not likely to be detected unless the proper calcium intake or absorption or both have been interfered with. These factors and a combination of others which include Ca/P ratio, acid-base balance, vitamin intake and the choice of the analytical method for blood or serum calcium undoubtedly explain the contradictory reports on mammals. Too many experimenters are willing to consider reliable the original Kramer-Tisdall (12) method for Ca, which involves its direct precipitation as oxalate directly from blood serum. The precipitation should be carried out on trichloroacetic-acid filtrates to insure the formation of pure calcium oxalate. Even with this and other improvements, the normal variations in blood-Ca concentration are ± 1 mg. per 100 cc. serum, whereas some investigators have considered a change of ± 0.5 mg. as significant.

A brief résumé of the studies on blood-calcium levels, as a result of gonadectomy, during menstruation and pregnancy and as a result of sex-hormone therapy in mammals, is given below and reveals the contradictory or insignificant results obtained by various investigators.

Gonadectomy. Blinoff (13), Hogben and Charles (14) and Cheymal and Quinquaud (15) find no changes in blood serum after gonadectomy in guinea pigs, rabbits and dogs respectively. These investigators recognize the normal fluctuations

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² The main results of these investigations were presented at the Sixteenth Annual Meeting of the Association for the Study of Internal Secretions, New Orleans, May 10, 1932.

in blood-calcium values. Decreases are reported by Dalsace and Guillaumine (16) in women, by Suzuki (17) and Okazaki (18) after ovariectomy in rabbits. Rises in blood calcium after gonadectomy were found by Leites (19) in rabbits and by Blanchetiere (20) in women. Leites reports results on only one male and one female by the Clark and Collip (21) modification for blood-serum calcium.

Menstruation. Boynton and Greisheimer (22) report falls in blood calcium during the menstrual or post-menstrual periods in normal women, but that no cyclic variations are observed in dysmenorrhea. These authors used the Clark-Collip modification of the Kramer-Tisdall method for serum Ca and give only the average values on a group of 12 women in the resting, pre-menstrual, menstrual and post-menstrual weeks. Bell (23) also reported lower blood-serum Ca just before menstruation. Widdows (24) found no changes during menstruation. Furthermore, Sherman, Gillett and Pope (25) and Bogert and McKittrick (26) found no change in Ca excretion during menstruation. Frei and Emmerson (27) found no changes in most cows during estrus. In one cow they observed a rise of 1 to 1.7 mg. Ca per 100 cc. prior to each of 3 estrus periods and a fall below normal several days later. The observations by Luckhardt and Goldberg (28) that parathyroidectomized dogs, which have been kept free from tetany by treatment, develop tetany during estrus are of interest and indicate a fall in blood-calcium level.

Pregnancy. Bodo and Liebmann (29), by the use of the frog-heart perfusion method, found no change in the concentration of ionic Ca in human serum as a result of pregnancy. Jansen (30), Mozocco and Moron (31) and Dennis and King (32) could find no significant difference in total serum-calcium in women during pregnancy. Meigs and associates (33) conclude the same in cows. Underhill and Dinnick (34) determined the Ca, Mg, K and Na content of human blood during pregnancy. They used the Kramer and Tisdall methods with trichloroacetic-acid filtrates on the laked blood. Their fluctuations were from 5.5 to 11.1 mg. Ca per 100 cc. of blood during pregnancy and 5.8 to 8.0 in another group of non-pregnant women. Nevertheless, they conclude that pregnancy leads to a rise in blood calcium. Many other investigators find irregular and slight lowering in serum calcium during pregnancy in women and cows (35, 36, 37, 38, 39, 40, 41, 42, 43, 44). No systematic attempt seems to have been made to study the same female before and during pregnancy with control of diet. Although in most cases the average values during pregnancy are slightly lower than for other normal females, nevertheless, the differences are of the same order or even less than the normal variations reported by the same investigators. In fact, Stander, Duncan and Sisson (40) regard the differences found by them insignificant and suggest a higher P/Ca ratio during eclampsia, which they associate with a higher inorganic phosphate rather than a lower calcium. Anderson (45), however, considers a value of 9 mg. per 100 cc., or less, as significant, as found in 82 per cent of 44 cases of eclampsia. Intravenous calcium therapy did not appear to relieve the symptoms.

Sex-Hormone Therapy. Mirvish and Bosman (46) call attention to numerous observations which indicate that the ovary plays a rôle in Ca metabolism and emphasize, as illustrations, the change in the skeleton after gonadectomy, the loss of Ca during menstruation, high blood-calcium after menopause and the supposed cure of osteomalacia as a result of ovariectomy. In fact, they assume a physiological antagonism between the parathyroids and the ovaries. They followed the serum-Ca changes in rabbits of both sexes as a result of the administration of crude lipin extracts from various organs and used the Clark and Collip (21) modification for serum Ca but probably precipitated from the serum directly. Their own observations as to the effect of ovariectomy in rabbits indicated no regular change in serum Ca.

Only one estimation was made before operation and 2 to 5 after, even as late as 2 months after the operation. The values varied from 13.9 to 18.0 mg. Ca per 100 cc. serum. A crude alcoholic extract, equivalent to 25 gm. ovarian tissue, injected subcutaneously, they report to lower the blood Ca by 30 per cent in 24 hours, although their normal values varied more than that. Larger doses by mouth gave similar results. No effects were observed from similar extracts prepared from muscle, pancreas, spleen, kidney and brain. Placental extracts also were inactive except in one case when the equivalent of 120 gm. fresh substance was used. The authors claim the same results on normal, pregnant, non-pregnant, mature, immature, ovariectomized and male rabbits. More purified extracts from cows' and pigs' ovaries and from cows' corpus luteum were injected into men and women. In most cases a fall in serum Ca was observed in 12 to 24 hours after one injection. The most striking case gave values of 11.3 and 10.3 mg. per cent before injection and 12, 24, and 48 hours after the injection 10.8, 7.4, and 11.2 mg. per cent, respectively. Their normal blood values varied from 9.6 to 11.6 mg. per 100 cc. serum. They also claim similar effects on male and female rabbits from similar extracts prepared from testis tissue and beef suprarenal cortical tissue, respectively. The results were very irregular, especially when 'more purified' preparations were used. No attempt was made to correlate this activity with estrogenic or other activities.

Reiss and Marx (47) injected rabbits with crude estrogenic material for 13 to 26 days and observed a fall of 1.7 to 3.5 mg. Ca per 100 cc. serum. Before injection the values ranged from 14.1 to 16.1 mg. Manzi (48), in spayed dogs, claims a rise in blood Ca after the injection of 'follicular hormone.' Mathieu and Barnes (49) report that both theelol and theelin lower the blood Ca in parathyroidectomized dogs but not in the normal animal. They found theelol more active than theelin. Dixon (50), however, found no relation between blood-calcium level and estrus, diöstrus, pregnancy, pseudopregnancy or ovarian and anterior pituitary hormone administration. He included dogs, rats and rabbits in his studies. A total dose of 6,000 M.U. of theelol injected in 2 days into young rats had no significant effect on blood Ca.

With anterior pituitary hormone, Cannavo (51) also obtained negative results on dogs, men and rabbits. Hogben and Charles (14), on the basis of very irregular results, conclude that the anterior pituitary pulp may cause a slight fall in blood Ca in rabbits and that following coitus a fall is also observed for some hours. Frei and Emmerson (27), however, consider a 0.5 mg. per cent rise significant following the injection of anterior lobe extracts into a cow in spite of normal fluctuations of 9 to 11 mg. per cent.

The brief review above led us to the conclusion that if the female sex-hormone does influence the blood-calcium level, it would appear that the fowl is the most suitable experimental animal. It was also hoped that more concentrated, more purified and biologically standardized sex-hormone preparations might give more uniform and significant results. We present our data and interpretations on fowl, rats and rabbits below.

Hormone Preparations. A). An estrogenic concentrate (UE) from human pregnancy urine was prepared and biologically standardized for us by Gustavson and D'Amour. This concentrate was diluted with olive oil as needed from time to time.

B). *Ovarian Extracts.* Pig ovaries were exhaustively extracted with boiling 95 per cent alcohol. This extract was evaporated under diminished pressure

and represents the 'crude extract' of Mirvish and Bosman (46). It is referred to as OE1. This extract is further purified by resolution in a limited volume of 95 per cent alcohol, chilled to remove some fats, phospholipins and cholesterol, evaporated under diminished pressure, dissolved in ether and phos-

TABLE 1. EFFECT OF SEX HORMONES IN FEMALE FOWLS, BLOOD-CA VALUES IN MG. PER CENT.

Date	Treatment	No. of Fowl						Min.	Max.	Average
		1	2	3	4	5	6			
7/27-30	Normal sample	8.2	6.1	9.2	8.0	—	7.3	6.1	9.2	7.8
7/29-30	Normal sample	11.1	9.4	18.5	7.3	8.8	10.8	7.3	10.8	9.3
7/30-31	Injected daily with UE. R.U.	5	5	10	10	15	15			
8/1		19.4	12.3	13.5	14.8	15.4	15.1	12.3	19.4	15.0
8/1-3	Injected daily with UE. R.U.	5	5	10	10	15	15			
8/4		17.0	12.3	13.3	—	12.5	13.0	12.3	17.1	13.7
8/5	Injected daily with UE. R.U.	5	5	10	10	15	15			
8/6		16.8	12.3	11.1	11.7	11.6	11.3	11.1	16.8	12.5
8/6-7	Injected daily with UE. R.U.	5	5	10	10	15	15			
8/8		16.4	14.3	11.6	12.9	10.0	12.5	11.6	16.4	13.0
8/11	Normal sample	15.7	24.1	13.6	11.2	11.4	14.3	11.2	24.1	15.0
8/13	Normal sample	14.0	21.0	21.8	12.5	11.8	21.1	11.8	21.1	17.0
8/15-16	Injected daily with 3 C.U. TAn									
8/17		14.5	14.1	13.3	13.3	12.9	14.6	12.9	14.6	13.8
8/17-18	Injected daily with 3 C.U. TAn									
8/19		12.5	14.7	15.7	14.4	12.7	—	12.5	15.7	14.0
8/19-20	Injected daily with 3 C.U. TAn									
8/21		14.7	10.5	11.8	12.1	11.6	11.8	10.5	14.7	12.9
8/21-23	Injected daily with 3 C.U. TAn									
8/24		12.7	10.0	12.5	9.1	9.3	13.3	9.1	13.3	11.2

pholipins precipitated by acetone. The acetone-ether solution was evaporated and the residue again dissolved in a small volume of alcohol, chilled and again taken through the acetone precipitation in ether solution. This product was practically free from phospholipin and cholesterol. It was evaporated and dissolved in olive oil to the desired concentration. An assay for estrogenic activity gave approximately 4 R.U. per cc. of final oil solution. The olive-oil solution

contained the equivalent of 25 gm. fresh tissue per cc. It is referred to as preparation OE.

C). *Male-Hormone Preparation.* This product was prepared by Dr. T. F. Gallagher from bull testis-tissue and contained 3 capon units per cc. It is referred to as TAn.

Analytical Methods. The blood (1 cc.) was drawn from fowl from a leg vein, from rats by heart puncture under light ether anesthesia and from rabbits by heart puncture without anesthesia. Unless otherwise indicated, trichloroacetic-acid filtrates were prepared from the whole blood. The Ca was determined by the Hauch and Koch (52) micro-modification of the colorimetric method of Roe and Kahn (53). For inorganic phosphate, a micro modification (52) of the Fiske-Subbarow (54) colorimetric method was applied on an aliquot of the trichloroacetic-acid filtrate.

EXPERIMENTAL OBSERVATIONS

Experiments on Fowl. Six young pullets and 6 young cockerels were divided into 3 groups each. Control blood samples were taken shortly after the noon hour, twice with a day intervening. Then, for 2 successive days, 5, 10, and 15 R.U. per 100 gm. body weight of preparation UE were injected into the respective groups. Then followed in sequence a day for taking samples of blood, 3 injection days, sample day, 2 injection days, sample day, 2 injection days, sample day. After 2 days of rest, the same series was repeated but 3 capon units of male-hormone preparation, TAn, was injected instead of the urinary female sex-hormone preparation.

The results are given in detail in tables 1 and 2. For the females, the blood-calcium values prior to any injection varied from 6.1 to 18.1 with an average value of 8.6 mg. per cent. For the males, the range was 6.8 to 11.5 with an average of 8.7. With the exception of the pullet which gave a normal value of 18.5, all the pullets responded with a higher blood calcium after treatment with 5 to 15 R.U.; even the lowest dose gave the maximum change; the range of Ca values for the pullets after the first 2 days' treatment was 12.3 to 19.4 with an average of 15.0 mg. per cent. All the cockerels also responded with a higher blood calcium ranging from 11.8 to 15.9, with an average of 13.3 mg. per cent. Repeated treatment with the same dosage of urinary female sex-hormone did not cause a further rise but rather a somewhat lower value in most cases. A 6-day period without injection did not return the blood calcium to the initial normal value. The cockerels reacted in practically the same way.

The effects from 3 capon units of male hormone daily were less obvious. The tendency was to lower rather than to raise the blood Ca. Inasmuch as the values still were distinctly above the initial figures, these observations are of little value. In fact, a subsequent experiment on pullets and cockerels also indicated that the male-hormone preparation in the dosage employed had no significant effect on the blood-calcium or phosphate levels. The average values indicate a rise in blood calcium and a fall in phosphate, but the individual birds did not react uniformly in the same direction and olive oil alone produced similar irregular changes. In fact, daily bleedings and analyses

TABLE 2. EFFECT OF SEX HORMONES ON MALE FOWLS. BLOOD-Ca VALUES IN MG. PER CENT.

Date	Treatment	No. of Fowl						Min.	Max.	Average
		7	8	9	10	11	12			
7/27	Normal sample	9.4	6.8	7.5	7.2	11.5	9.4	6.8	11.5	8.6
7/29	Normal sample	9.4	—	7.2	8.8	9.4	9.4	7.2	9.4	8.8
7/30-31	Injected daily with UE. R.U.	5	5	10	10	15	15			
8/1		12.4	12.5	14.3	12.7	11.8	13.9	11.8	15.9	13.3
8/1-3	Injected daily with UE. R.U.	5	5	10	10	15	15			
8/4		11.4	11.8	11.1	11.2	10.4	10.3	10.3	11.7	11.0
8/4-5	Injected daily with UE. R.U.	5	5	10	10	15	15			
8/6		8.7	11.1	9.6	9.5	10.7	10.3	8.7	11.1	10.0
8/6-7	Injected daily with UE. R.U.	5	5	10	10	15	15			
8/8		10.0	12.3	12.5	10.7	12.5	10.0	10.0	12.7	11.4
8/11	Normal sample	11.8	10.0	10.5	11.1	13.3	13.3	10.0	13.3	11.7
8/13	Normal sample	16.7	11.8	11.8	11.3	11.8	11.6	11.3	16.7	12.5
8/14-16	Injected daily with 3 C.U. TAn									
8/17		10.3	13.1	11.1	12.4	10.0	11.1	10.0	13.1	11.7
8/17-18	Injected daily with 3 C.U. TAn									
8/19		14.4	12.5	14.0	10.4	12.5	12.5	10.4	14.4	12.7
8/19-20	Injected daily with 3 C.U. TAn									
8/21		11.4	10.5	10.0	11.0	10.3	11.3	10.0	11.4	10.7
8/21-23	Injected daily with 3 C.U. TAn									
8/24		11.0	10.0	10.4	13.3	10.0	—	10.0	13.3	11.0

for 2 successive periods of 3 days each also gave fluctuations of 8.1 to 11.6 mg. as average Ca. values for a group of 7 hens and 7 cocks. The fluctuations for an individual bird were, however, much greater in some cases and no sex difference was observed.

The following summer the experiments on young fowl were repeated with estrogenic hormone and with adequate untreated and olive-oil injected controls. The results are given in table 3. The blood-Ca values are so very irregular for treated as well as for untreated fowls that no significance can be attached to the changes observed in the 2 previous experiments. The 2 uninjected fowl fluctuated between blood-Ca values of 12.2 to 20.5 and 10.4 to

16.7 mg. per cent respectively. The 3 olive-oil injected fowl varied from 11.4 to 16.0, 10.5 to 13.1, and 10.6 to 17.4 mg. respectively; after 4 daily injections of 200 R.U. of UE no significant change was observed. Only 1 of the 3 UE-injected fowl responded with a distinctly higher blood Ca than during the preliminary control period. The other 2 showed practically no change after 3 daily injections but after further treatment changed to the preliminary

TABLE 3. ACTION OF URINARY ESTROGENIC HORMONE ON FOWLS. BLOOD Ca AND P, IN MG. PER CENT.

Date	Treatment		No. of Fowl							
			Injected with 200 R.U. of UE			Injected with 1 cc. Olive Oil			No Injection	
			157	158	159	154	155	156	152	153
7/18	Normal sample	Ca	26.7	15.7	12.4	14.8	12.9	16.0	20.5	12.9
		P	4.8	4.2	3.6	3.3	4.2	4.8	5.5	3.4
7/20	Normal sample	Ca	22.2	21.3	10.0	16.0	13.1	12.6	16.7	13.2
		P	4.8	4.2	3.8	3.4	3.8	3.5	4.3	4.0
7/21-23	Injected daily									
7/23		Ca	22.2	22.2	20.0	16.0	11.8	13.6	14.8	11.1
		P	4.6	5.0	4.5	3.9	3.2	3.0	3.2	4.0
7/24-25	Injected daily									
7/25		Ca	17.8	21.0	21.8	11.4	10.5	17.4	16.0	10.4
		P	4.8	4.7	4.7	4.6	3.2	3.8	4.3	4.1
7/26-27	Injected daily									
7/27		Ca	14.3	15.4	19.4	12.1	11.7	10.6	14.8	11.9
		P	3.7	4.0	5.3	3.0	3.1	2.9	4.3	4.1
			Injected with 1 cc. Olive Oil			Injected with 200 R.U. of UE			No Injection	
7/28-30	Injected daily									
7/30		Ca	12.9	18.2	22.2	13.4	13.5	14.3	12.2	16.7
		P	4.7	4.6	5.9	3.5	3.3	2.9	3.5	3.3
7/31	Injected									
8/1		Ca	13.2	17.4	19.4	10.8	13.4	11.3	15.4	11.1
		P	4.5	4.5	4.3	3.4	3.4	2.9	2.9	3.6

control level or lower. The inorganic phosphate values varied only slightly. From these observations on such high doses of estrogenic material, we conclude that the urinary estrogenic hormones do not affect the blood Ca in fowl. The rises observed in experiments 1 and 2 no doubt were due to normal fluctuations or to the effect of oil on the original calcium values which were very much lower than in this experiment. In tables 1 and 2, the preliminary control values ranged from 6.1 to 11.5 while in table 3, the range was 10 to 26.7 mg. per cent.

Further attempts to control the normal fluctuations in blood-calcium level in fowl were made. Seven pullets and 8 cockerels were bled 7 times in the course of 10 days. The food intake was controlled and the animals were

caught and placed in small cages some hours before bleeding in an attempt to minimize excitement. The same methods for calcium and inorganic phosphate were used, but special precautions were taken to control the time during which the trichloroacetic acid was in contact with the precipitated protein before separating by centrifuging. The blood-Ca level fluctuated between 7.5 and 14.3 mg. per cent in the females as a group and between 6.5 and 13.8 in

TABLE 4. EFFECT OF BLEEDING AND ESTROGENIC HORMONE ON Ca AND P LEVELS IN ALBINO RATS. VALUES IN MG. PER CENT.

Date	Treatment		Olive Oil Injected			Hormone Injected									
			Female	Males		Females			Males						
			12	3	8	2	9	10	2	4	5	7	9	10	
2/7	Uninjected	Ca	7.6 4.7	6.1 5.0	7.6 3.4	— —	5.4 4.7	9.8 4.0	5.7 3.5	7.5 4.2	8.8 4.5	6.6 5.4	5.4 4.7	5.4 5.5	
2/8	Uninjected	Ca	11.1 5.7	— —	8.3 5.6	— —	6.7 5.3	8.7 4.9	6.6 5.0	8.9 4.8	8.1 5.1	— —	4.7 5.1	— —	
2/9	Uninjected	Ca	10.9 3.9	6.2 6.0	6.2 4.1	7.4 4.7	8.7 4.7	— —	8.5 4.8	8.5 4.1	— 4.8	— —	7.2 4.4	— —	
2/10	Uninjected	Ca	9.1 4.7	— —	8.1 —	— —	— —	— —	— —	8.5 4.6	7.3 5.1	7.8 5.1	— —	7.7 5.9	
2/11	Uninjected	Ca	7.7 5.6	6.2 5.8	— —	9.4 4.9	5.8 5.7	8.4 5.3	6.9 5.1	7.6 5.3	10.3 6.7	6.0 5.1	6.5 6.0	7.4 6.7	
2/12	Uninjected	Ca	7.9 5.6	6.9 4.6	6.7 —	7.6 5.0	6.0 5.5	7.1 4.6	9.7 5.2	9.5 6.7	7.1 5.9	7.1 5.0	— —	9.8 5.2	
2/13-15	Injected		1cc. olive oil daily						10 R.U. of UE daily						
2/16	Uninjected	Ca	10.2 4.0	5.7 4.4	7.9 3.1	9.0 4.8	7.0 4.4	8.2 5.2	7.7 4.1	7.9 3.1	10.0 4.0	8.6 5.2	7.1 4.2	— —	
2/16	Injected		1 cc. olive oil						10 R.U. of UE						
2/17	Uninjected	Ca	8.4 5.1	7.9 5.3	9.5 4.7	7.0 3.8	5.7 5.5	6.6 4.8	7.0 5.0	7.0 4.5	6.7 4.6	6.5 5.3	6.7 5.3	6.9 6.2	
2/17	Injected		1 cc. olive oil						10 R.U. of UE						
2/18	Uninjected	Ca	10.5 5.0	8.2 4.7	9.0 4.0	7.1 3.1	8.3 4.3	6.2 3.6	7.0 4.3	9.4 4.3	7.1 4.3	7.0 4.6	9.6 4.3	7.4 5.3	

the males. The least variation in individual fowl was 2.1 mg. per cent in females and cockerels each; the maximum variation was 4.7 in an individual pullet and 6.1 in an individual cockerel. Furthermore, after some of these fowl had been allowed to rest for 2 weeks, the blood-Ca values were again determined before and after the 2 daily injections of 10 R.U. of UE and olive oil, respectively. No consistent changes were observed. The fluctuations were in both directions and of the same order as during the long control period. Other fowl of the original group were allowed to recover for 24 days after the preliminary control period. After again determining the normal Ca level, 4 fowl were injected for 2 days with olive oil and 7 for 2 days with 50 R.U. of UE. The olive oil as well as the hormone-injected animals all responded

with slight falls in blood-calcium values. The original values in these fowl were of the order of 8.0 to 11.8 mg. per cent, so that we might have expected a rise as in the first experiment where the original range was 6.1 to 11.5 (tables 1 and 2). Old hens with blood-calcium levels at 13.8 to 24.9 responded equally irregularly to olive oil and urinary estrogenic injections. We are at a loss to explain these discrepancies. However, the normal fluctuations in blood Ca and the irregular results from high doses of urinary estrogenic

TABLE 5. EFFECT OF WHOLE OVARIAN EXTRACT (OE) ON BLOOD Ca AND P IN NORMAL AND GONAD-ECTOMIZED ALBINO RATS.

Date	Treatment		Females						Males					
			Normal			Spayed			Normal			Castrated		
			Hormone treated 31 51		Con- trol 24	Hormone treated 2 10		Con- trol 3	Hormone treated 11 45		Con- trol 1	Hormone treated 2 513		Con- trol 413
4/13	Uninjected Ca P	7.1 6.8 4.3 4.6	7.9 3.8	6.3 5.7 4.7 3.4	6.3 4.0	7.5 6.8 4.6 4.6	6.6 4.3	5.3 4.7 3.5 3.0	6.3 3.3					
4/14	Uninjected Ca P	7.7 5.8 5.0 4.8	6.0 4.2	6.0 5.4 4.8 4.2	5.4 3.8	5.7 5.4 4.8 4.4	7.1 4.4	6.3 5.4 4.1 4.1	6.7 3.9					
4/15	Uninjected Ca P	6.7 7.8 4.4 4.4	6.7 3.4	6.4 5.8 4.3 3.3	5.5 3.8	7.5 6.5 — 4.4	6.8 4.0	— 5.4 3.3 3.1	7.0 3.5					
4/16	Injected with	OE	olive oil	OE	olive oil	OE	olive oil	OE	olive oil					
4/17	After 24 hr. Ca P	7.3 7.1 5.3 5.3	6.4 4.6	9.3 8.0 5.1 4.6	6.5 4.0	6.6 6.3 5.5 5.7	5.8 4.4	6.5 9.2 4.1 4.1	6.8 3.9					
4/17	After 36 hr. Ca P	10.9 9.1 5.4 4.9	7.3 4.4	6.4 7.9 4.1 4.9	7.6 4.3	8.8 10.0 5.7 6.5	8.5 4.6	7.3 10.0 4.0 4.7	7.7 4.3					
4/18	Ca P	7.5 9.3 5.0 5.6	8.4 4.4	10.3 8.0 5.4 4.5	8.2 4.4	7.6 8.6 4.6 6.7	8.8 4.8	7.9 9.1 4.5 3.7	6.1 3.8					
4/19	Ca P	7.8 7.7 4.9 4.8	7.2 3.8	8.8 9.7 10.0 3.9	7.8 4.1	7.3 8.1 4.0 5.5	7.6 4.1	7.3 8.7 4.0 3.7	6.3 3.8					
4/20	Ca P	7.1 8.3 4.5 4.8	9.1 4.5	— 7.7 — 4.4	7.8 3.8	6.8 7.2 4.6 5.7	9.7 5.1	7.1 7.0 4.4 4.0	6.2 4.6					

material lead us to conclude that there is no satisfactory evidence that the estrogenic material affects the level in fowl.

Experiments on Rats. In order to determine the effect of daily heart punctures and light ether anesthesia on the blood-Ca level in rats, 12 animals were bled by heart puncture under light ether anesthesia for 6 successive days and the blood calcium and inorganic phosphate estimated by the methods previously referred to. The greatest fluctuation in blood-calcium values in an animal was 5.7 to 9.7 mg. per cent. In the other 11 rats the fluctuations were less, but the most constant blood Ca in one animal fluctuated between 6.1 and 6.9. Obviously the variations in rats are of the order of 0.7 to 4.0 mg. per cent. The results are given in table 4.

Three of the animals above were next injected with olive oil and the others with 10 R.U. of UE dissolved in olive oil. The effect on blood Ca was very irregular in both groups of animals and the changes were of the order observed prior to the treatment. In fact, the animals which showed the greatest fluctuation before injection also did so after the olive oil and estrogenic hormone injections, respectively. Study of table 4 leads to the conclusion that no significant change in blood Ca was brought about by the injection of estrogenic hormone.

In order to compare our studies with those of Mirvish and Bosman (46), we also studied the effects from a concentrate of the estrogenic fraction from

TABLE 6. EFFECT OF WHOLE OVARIAN EXTRACT (OE) ON BLOOD Ca AND P IN RABBITS.

Date	Treatment		Females				Males		
			Hormone treated		Controls		Hormone Treated		Control
			2	3	1	4	1	4	2
4/29	Uninjected	Ca	11.4	11.0	10.4	11.0	10.5	10.3	12.1
		P	5.9	5.8	5.8	5.7	5.6	6.8	6.2
5/1	Uninjected	Ca	11.4	10.5	10.3	11.1	10.8	10.5	10.8
		P	6.7	5.8	6.1	5.9	5.3	5.8	5.9
5/3	Uninjected	Ca	10.6	10.0	10.3	9.1	11.4	11.1	10.7
		P	5.9	5.8	6.7	5.8	5.8	6.3	5.9
5/4	Injected with		OE		Olive oil		OE		Olive oil
5/5		Ca	13.3	11.9	12.9	10.2	12.3	13.8	11.1
		P	5.6	5.4	5.4	5.7	4.2	5.6	4.6
5/6		Ca	11.9	13.0	18.2	14.8	11.1	15.7	15.7
		P	5.7	4.9	5.0	5.7	5.5	5.5	5.4
5/7		Ca	9.5	12.5	9.8	10.9	10.0	12.9	12.9
		P	5.0	5.5	5.2	5.9	5.3	6.2	5.6

hogs' ovaries. The preparation OE described above was injected for these purposes in doses equivalent to 25 gm. original tissue. The results of these studies on normal and gonadectomized rats are given in table 5. The results on the normal animals confirm the observations with the urinary estrogenic preparation. However, the gonadectomized animals appeared to have somewhat lower blood-Ca values prior to the treatment and the injection of OE equivalent to 25 gm. fresh ovary tended to raise this level. The olive oil did this to a less extent. Before this can be stated to be a fact, more extensive experiments are needed for confirmation.

Experiments on Rabbits. Rabbits were injected with OE in doses equivalent to 25 gm. fresh ovarian tissue and controls were injected with olive oil. In the first experiment the blood drawn by heart puncture without anesthesia was analyzed for Ca and P by the methods used above. The results are given in table 6. The results are contrary to those reported by Mirvish and Bosman

(46). In all cases a rise in blood Ca was obtained and olive oil appeared to be about as efficient as the hormone preparation. The inorganic phosphate tended to become lower in all cases.

In the second experiment on rabbits, the same hormone dosage was used but Ca was estimated in the serum by the Tweedy-Koch (55) modification of the Kramer-Tisdall method (12) and not on trichlor filtrates from whole blood. The results on 2 controls with olive oil and 4 with OE injections are given in table 7. It is obvious that the changes in blood calcium are neither consistent nor significant.

DISCUSSION

Our survey of the literature calls attention to the contradictory claims concerning the relation of blood-calcium level to ovarian activity. It is generally agreed that marked and sudden rises in blood Ca are associated with the

TABLE 7. EFFECT OF WHOLE OVARIAN EXTRACT (OE) ON BLOOD-Ca LEVEL IN RABBITS. VALUES IN MG. PER CENT.

Date	Treatment	Females				Males	
		Hormone Treated		Controls		Hormone Treated	Control
		1	4	2	3	2	1
5/19	Uninjected	12.1	15.6	15.4	15.4	14.2	15.0
5/22	Uninjected	15.4	15.2	15.7	16.0	15.2	15.6
5/25	Uninjected	13.6	15.4	14.9	15.4	15.3	15.4
5/25-27	Injected daily with	OE		Olive oil		OE	Olive oil
5/28		15.0	15.0	15.1	15.0	15.1	15.3
5/28-6/1	Injected daily with	OE		Olive oil		OE	Olive oil
6/3		16.1	14.9	16.8	16.4	15.9	16.5

calcification of the egg shell in the dove, pigeon and fowl. How this mobilization of Ca is controlled is not known. From our experiments on fowl, we conclude that we have not been able to associate this rise consistently with an estrogenic activity.

In mammals the need for extra calcium mobilization during pregnancy is also present, but the periodicity of this need is not the same as in birds. The observations on blood-calcium levels in pregnancy have led investigators to claim *a*) that no effect is observed, *b*) that a rise occurs, and *c*) that a fall results. As previously stated, it is probable that proper control of the Ca intake and of other dietary factors will prevent this fall in blood calcium in many cases. It is also conceivable that individuals may possess idiosyncrasies in Ca metabolism and thus yield abnormal values in either direction during pregnancy. To assume that an ovarian hormone controls the level in pregnancy is not well-founded. Nevertheless, Mirvish and Bosman (46) associate ovarian hormone activity with the supposed fall in blood Ca during pregnancy be-

cause they report a fall after the use of their crude ovarian extracts on rabbits and women. We have not been able to confirm these findings on rats or rabbits with urinary or ovarian estrogenic preparations. We have had some suggestions of a rise in blood Ca from such treatment, but the rises are irregular and insignificant as compared with the olive-oil injected controls and with the normal fluctuations observed without injections. Even if we grant that a slightly lower blood Ca is observed in pregnancy, this does not logically lead to the conclusion that the fall is due to an ovarian hormone. The real cause would appear to be the foetus. If the ovarian hormone were to lower the blood Ca without the presence of the embryo, as Mirvish and Bosman (46) claim, the conditions would be very unfavorable for both mother and foetus. It would seem more logical to expect a rise in order to protect the pregnant mother and to aid the foetus. Our observations on the gonadectomized rats appear to lend some support to this possibility because we observed a slight lowering of blood calcium as a result of gonadectomy and a subsequent suggested rise after the injection of the estrogenic material in both sexes. In these studies, the olive-oil injections produced very little and irregular changes in the level. Before these suggestive findings are accepted as proof of hormone action, further studies must be conducted on more animals and with the pure crystalline sex hormones. These were not available when our experiments were carried out.

SUMMARY

The normal blood calcium in fowls fluctuates from 6.1 to 26.7; in rats from 4.7 to 11.1; and in rabbits from 9.1 to 16.0 mg. per cent. Frequent daily bleedings do not appear to be responsible for consistent changes in the level.

A non-crystalline purified estrogenic product prepared from pregnancy urine did not produce a significant and consistent effect on the blood-Ca level in fowls, rats and rabbits. A non-crystalline purified estrogenic preparation from hog ovaries yielded the same results. When gonadectomized rats were studied, the results suggested a slightly lower blood-Ca level than normal and a tendency to a rise after the injection of the ovarian estrogenic preparation. A male-hormone concentrate prepared from bull testis-tissue also produced no change in the level in fowls.

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